IN VITRO CELL MIGRATION QUANTIFICATION: APPLICATION USING LABVIEW FOR SCRATCH ASSAYS

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INTRODUCTION

The wound healing or scratch assay is an easy, low-cost and well-known method to measure cell migration in vitro (Liang et al 2007). Scratch assay is conducted in order to study directional cell migration and cell-cell interaction in vitro. The basic procedure involve creating a “scratch” in a cell monolayer, capturing the images at the beginning and at regular intervals during cell migration to close the scratch, and to quantify the migration rate of the cells. Besides monitoring migration of homogenous cell populations, this method has also been adopted to measure migration of individual cells in the leading edge of the scratch.

The main disadvantage and limitation of the in vitro scratch assay are the procedures used for image analysis. Image Pro-Plus software (Media Cybernetics) or a freeware (http://rsb.info.nih.gov/ij/) are frecuently used. Obtaining statistically significant results on cell migration requires time-consuming analysis of images due to the necessity to adjust parameters manually.

OBJECTIVES

Our goal is to develop an user-friendly, LabView based software, to accurately analize scratch assays images, easy to be used without programming skills, fast and automated.

MATERIALS AND METHODS

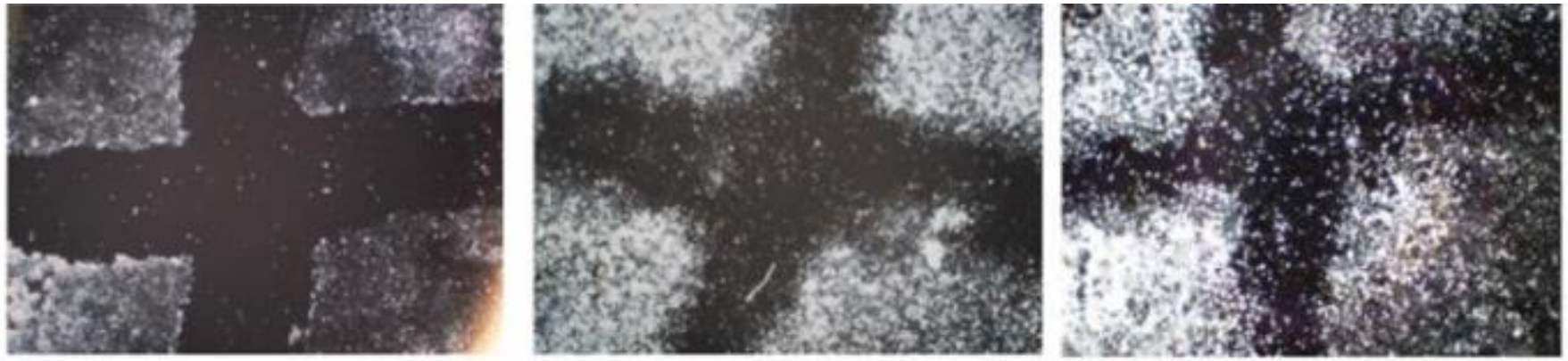
Cell Cultures

LNCaP cells (American Tissue Culture Collection, Rockville, MD) were routinely cultured in RPMI growth media supplemented with 10% FBS (Invitrogen). The cells were sheeded on sterile coverslips and let to growth until 70% confluence.

Scratch assay procedure

The cell monolayer was scratched with a p200 pipet tip, creating cell-free cross lines (Fig 1). The debris were removed, the cells were washed once with growth medium, and the culture medium was changed. Then, the first contrast pase micrographs were taken (day 0) The cells were mantained at the incubator for 24h and then were again micrographed, then cultured for 48h and micrographs were then obtained (72h total culture time).

Fig.1



LNCaP day 0 | LNCaP 24h | LNCaP 72h

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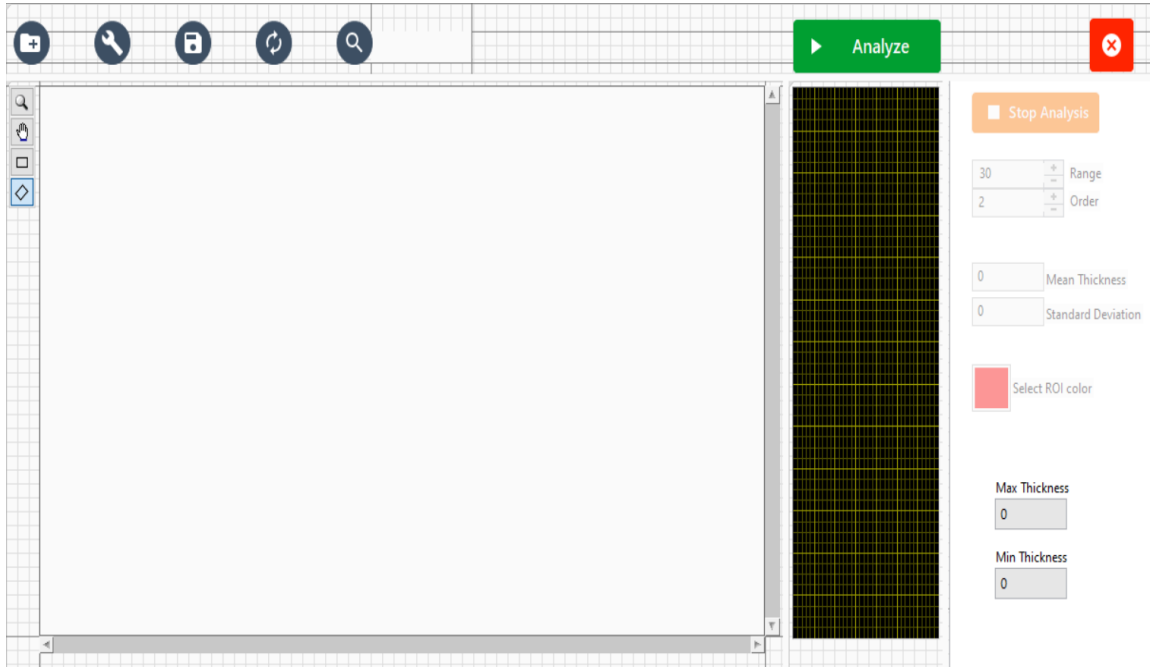
LabView

LabView software has two components for programming a front panel and block diagram. The front panel is an interactive part where the user can enter data and has an interface área with different visual elements. Figure 2 shows the front panel of the program which has been developed for scratch assays.

The block diagram is the place where the actual Programming operation was done. The source codes are run in this place which is created by using the links between virtual objects. Post-processing outputs can be seen on the front panel software input and output processing. All virtual elements which have input and output ports can be seen as an icon. The block diagram is created by using this icon combination for software development. Figure 3 shows the block diagram which was developed by using LabVIEW platform for scratch assays.

Fig.2:
Front panel



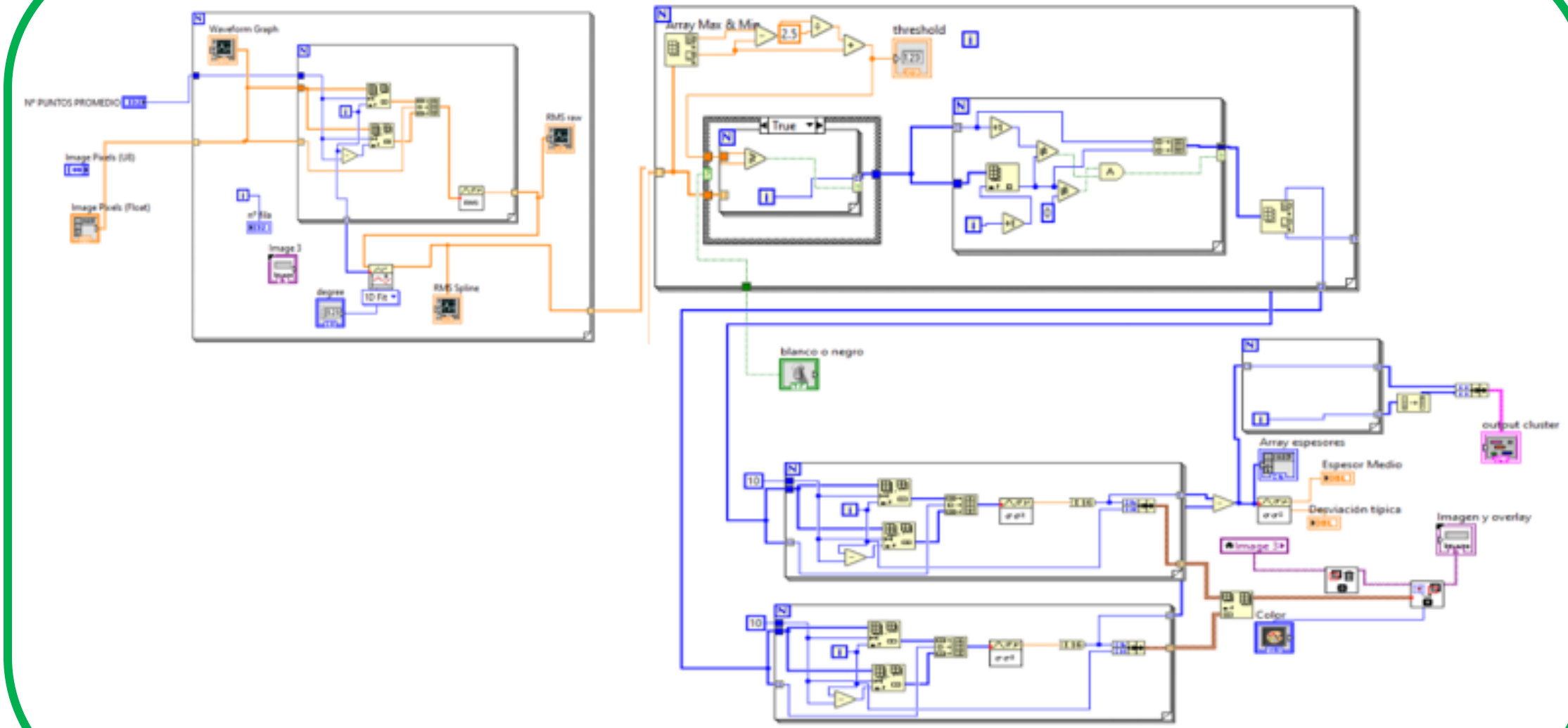
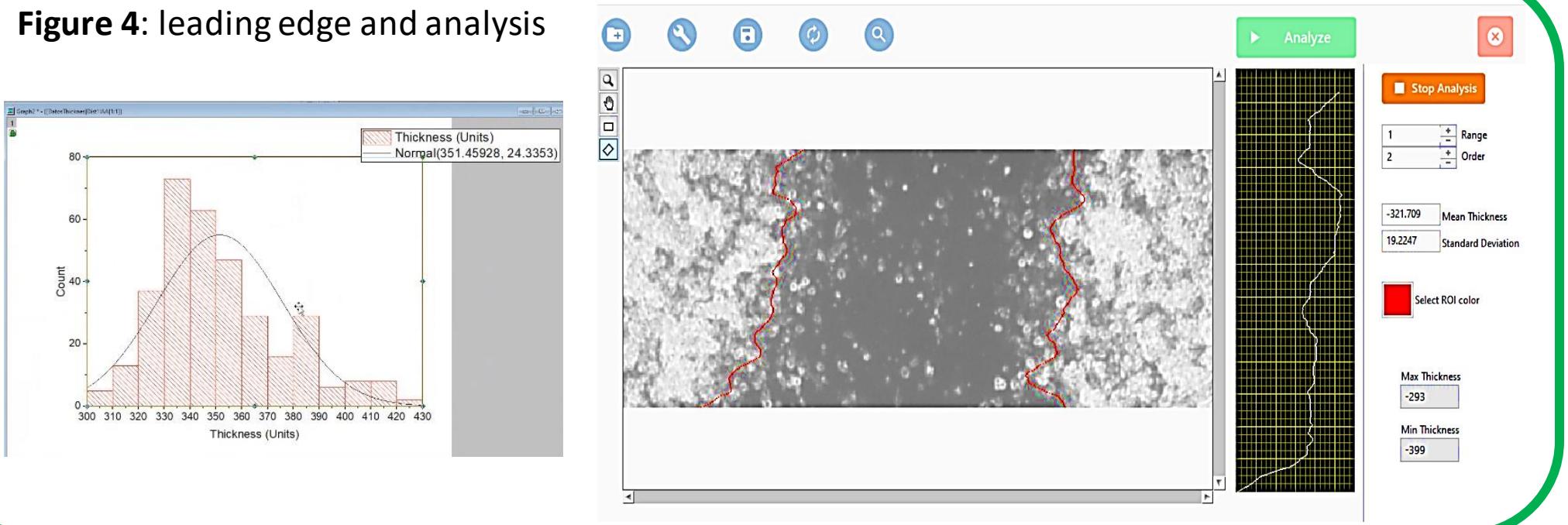


Figure 3: block diagram

RESULTS

We used the application developed to locate the position of the leading edge of the wound and to calculate the wound area in all experimental images. To provide some qualitative comparison of the detected leading edge we superimpose on the experimental images the leading edges detected by the application with the leading edge manually detected by an experienced scientist. Visual interpretation of the position of the detected edges suggests that the edge detection application clearly and accurately detects the edge of the scratch in all assays we analyzed. Moreover, to quantify the progression of the migration we measured the area enclosed by the detected edge, and examined how this area decreases with time as the wound closes in time-lapse images. We compared our results with those obtained by the ImageJ edge detection algorithm, and concluded that our application generate accurate results.

Figure 4: leading edge and analysis



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CONCLUDING REMARKS

Wound healing assay is a widely used method to evaluate cell migration. However, obtaining statistically significant results requires time-consuming analysis of hundreds of images. This analysis is usually made in the semi-quantitative manner, and results are compared after large time intervals (Wang et al., 2019). Software tools increasing quantitative output through automated image segmentation have been suggested, such as TScratch (Gebäck et al., 2009), AIM (Cortesi et al., 2017), and ImageJ tools (Baecker, 2012; Nunes and Dias, 2017; Suarez-Arnedo et al., 2020). Up to date, none of them becomes widely used for high-throughput study of wound healing due to the necessity to adjust parameters manually.

Our image analysis application is actually under development with the goal to generate accurate and reproducible results for wound healing assays on phase contrast images.

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